Concerning Hayes' paper in Nature:

Have similar experiments been done with one parent lysed with phage? Of course I realize that this is not strictly comparable to the strep experiments. M. Vogt was thinking of doing this but whether she did or not I was never able to find out.

Concerning Cavalli Maccacarro in Nature - i.e., that a hi frequency of recombination is obtained when the supernatants of the washed cultures are mixed (concluded to be due to residuum of non-sedimented bacteria plus growth factors allowing plate microcolony formation):

a. the residuum of non-sedimented cells would probably be single cells rather than clumps (which form when 0.9 % is added to effect the secomd washing) hence there is a greater chance of cells of different parentage coming into contact than in clumps thus giving more recoverable progeny

b. M. Vogt, when she decided not to follow the methods I used to produce kinetics results, would grow both parental strains in NB to about 10 to the 8th, mix equal volumes, allow several divisions to occur (grow together one hour), then plate, without washing, various volumes in minimal medium, the total volume plated being 1/2 of the total volume of the system which was then made up by adding fresh NB (in this manner she thought that the cells in the cross were being held in a steady state as far as the environmental and cell conditions were concerned)

Obviously the recovery of prototrophs will be proportionally greater, due to carry-over of brovth which is not used up, when a larger volume of inoculum is plated, due to microcolony formation and plate recombination.

In looking over Peg Lieb's letter in which she says she crossed K12 S X W1177 I note that she has performed the cross both ways - by my method with the result that the protos are proportional to the product of the parent concentrations, and by the Vogt method where the protos are proportional to the sum of the parents plated. Apparently ML doesn't realize that this is just as it should be and to get product relationship she should run several tubes with varying concentrations of parental cells. Growth of course louses up the data - one has to apply the theroem of mean value and then too there is the problem of segregation and division of the prototrophic segregants with math similar to the mutation and growth of mutant problem.

Further concerning Hayes:

(1) specific objection

Since there are no methods nor no data given in the Nature paper it is difficult to make any criticism but I wonder about the following possibility: (a) crossing was performed by mixing 58-161St (strep treated) with W1177 in minimal medium (I suppose) while (b) the sterility of the 58-161 St (or the amount of killing) was determined by plating an aliquot in strep-free agar, and probably nutrient agar either before or after washing with saline (makes little difference since a bacteriocidal amt of strep can remain attached to cell and cannot be washed away according to some bact 'physiologists') since 58-161 is auxotrophic. Now killing may be (and is according to some work I looked into and the bact physiol people) different for minimal and nutrient media. Thus Hayes is not actually testing the viability of the 58-161 cells used in the cross. Actually the cross should be run in liquid and plated (a) in minimal for recombs, and using kinetics expressions, and (b) in enriched (with proper controls to determine differential killing in minimal and enriched to determine residual viable 58-161 cells) as a function (time).

(2) Baldo (Arnold Ravin - of Alcaligenes fame) writes from Paris where he is working with Harriett Ephrussi-Taylor on "mapping the transforming principle (TP)" - see ECR for ET's theoretical paper (and that's all it is to me - until I see quant data) - and working out effect of agar, complex pneumo medium, culture phase, etc. (bact physiology) on frequency of transformation. They have just heard of Hayes work and think that it explains all of Lederberg's results ("better than L's original hypothesis") and mine ("I imagine your kinetic studies are much more understandable in these terms than in terms of a complicated sexual process"). H's hypothesis being of course that genetic material is carried on surface of corpses of lysed K12 cells). I pointed out that:

(1) One should beware of explaining all phenomena in terms of one's own specialty - apparently the Paris people think that the genetic factors are really TP's stuck to the cell wall.

(2) How does one explain - linkage (remembering that ET's hypothesis concerning TP interaction is that and only that), heterozygous diploids, cytology (Baldo saw the slides in 49-50 of diploids that you had at CU)?

(3) Crosses between K12 lysogenic (W1177) and K12 non-lyso had been carried out and probably between two non-lysogenic auxotrophic substrains (yourself? Esther? I think I heard something about Marguertite Vogt's doing it too). as well. If W1177 must accept gametes from some other strain then one certainly is up a tree in thinking that lysis is necessary since apparently none will occur with K12S since no lambda is present. (Baldo brought up explanation of Texas effect in terms of lysis and release of lambda).

I hope this quells the Paris sentiment that "This is terrific stuff and I am convinced as is everybody else in Paris that "sexuality" is an incorrect explanation for bacterial recombination."

Concerning the s/ paper - I got your remarks and puzzled them out on a camping trip over the week-end, by the aid of a flashlight in the tornado's tail:

(1) origin of K12 s-: It was the stock that Ryan gave me as K12. Of course it was reisolated several times from a single cell. The use of EMS is OK - both s- and s/ will grow on it - the s- not as rapidly or as luxuriently nor will they reduce Tphenyl tetrazolium compounds as well. However I am willing to reword sections of the paper to let the onus of identification fall upom the s-.

(2) "classical coliform" is Bergey's. Caginess re contam is FJ's doing. OK by me to cut table II - plating of s/ and s-.

(3) temp sensitive mutant reference can be reworded

(4) and (8) Wide variations in oxidation rates exist for log phase and stationary phase cells - but using the log cells the variation is less and significantly different in s/ amd s-/ Of course manometric analysis is a necessary evil if cell-free preps are not used (I had no TPN to measure the reduction of at 340 mu in the Beckmann - malic enzyme and dehydrogenase are TPN specific in coli according to Korkes of Ochoa's gang). But, as you say, it is fruitless in explaing the nature of the block and doesn't analyze the position, exactly, of the block, that's the rub. I doubt if all the reaction steps of the Szent-Gyorgyi scheme have been worked out - high energy phosphate is known to be generated but no intermediates have been isolated that are phosphorylated.

(5) so/ might be a residual original K12 - tho' the original K12 went thru several single colony isolations

(6) Thanks for the reprint. I have it, your diploid, and the T and L J Bact. 53 papers. True, s- [redacted] might have slipped in but it wasn't my fault since 2 mutants, prepared prior to my obtaining the culture, were prepared (A-3 and B-6) and are s-. s- is lysogenic I believe - Peg Lieb tested it for me by UV lysis and plating on S.

(7) Please - my original gebiet and you won't let me mention it. So what if Delbruck's person couldn't repeat it? You know who it was (grapevine says she tried to repeat Hayes stuff and couldn't). Besides Delbruck is reported to have been telling people "Unfortunately we have been able to repeat Nelson's work" tho' I must check on this from more reliable sources. Knowing D. I'm sure this is not a misquotation since he would never say 'Unfortunately we have been /illegible/ unable to repeat Nelson's work". In any cross I use kinetics methods just to check that I'm getting crossing.

I'm willing to include the genetics but how to report it? All it is is numbers and if you can figure a consistent linkage you're good. If I'd only known about the replica technique when I did this!

The paper is padded considerably - Mitchell wants it shorter and Ryan longer (and cagier). Trying to serve several masters at once is difficult. Trouble is when I talk genetics in genetic shorthand Mitch wants it expanded and when I talk chem in chem shorthand Ryan wants it expanded.

Your recent letter just arrived re the F/. There seems to be a whole new business opening up.

Concerning the light workmsee the ff attached experiment. The rates (K in the kinetics equation) were somewhat lower at Tech than CU so I decided to see what happened under illumination. The cross is run between 679-680 and Y24 using the standard plate technique - 5ml total volume is mixed on 10 ml of agar-salts in a Petri (no glucose) - allowed to stand for a varying length of time for different plates and then 10 ml of 1.5 X concentrated agar-salts-glucose added. One series was kept in dark and the other exposed to a battery of fluorescents. The difference is obvious. Now comes work on the mechanism:

(a) is it the texas effect? probably not since the filter consists of several layers of iron glass as well as pyrex and fluorescents give off little UV. Conversely is the texas effect this? I don't know.

(b) is it infra-red and increase in chiasma frequency? Probably not since Y24 X p gives increase. I must test other crosses and see if linkage values are the same in light and dark before the final answer. But the filter includes a copper sulfate filter to take care of IR.

(c) Is effect upon rate or upon saturation level? Upon rate but may also affect sat level, don't know yet since I haven't hit a cross with saturation yet.

(d) Is effect temp difference? Probably not - Q10 run now being made but dark controls only 1.8 degrees lower (this means Q10 of about 10) in given experiment and in later table was heated in dark controls by flame below to give slightly higher temp.

(e) Is effect due to triggering something in cells or must light be supplied continuously? Don't know - must irradiate cells before mixing to answer this.

(f) Is effect due to release of something into medium? (Lysis may be caused by visible but viable count remains the same yet this is not proof positive since only syngamable cells may lyse and wouldn't be detected). Haven't tested culture filtrates yet. If something is thrown off then comes the biophysics (action spectrum) and the chemistry - Kuhn, here we come.

So far I've applied only to Merck. USPHS is next if Merck says no but it would be best to wait until June 15th deadline since this set is decided just after (and not before) the new USPHS budget is granted July 1. The application states Sept. 1, 1952 - thought we could always modify this.

Sorry to have written a book.

Regards,

Jim C